

APPLN. NO.: 10/659,367

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RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

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**AMENDMENTS TO THE CLAIMS:**

Please amend the claims as set forth below.

1. (Currently Amended) An analytical device comprising a porous material that permits liquid to migrate therein, the device comprising in the migration direction:
  - (i) a first zone onto which a sample suspected of including an analyte to be assayed can be applied,
  - (ii) a second zone comprising a non-immobilised molecule capable of specifically binding to the analyte, said non-immobilised molecule including a detectable label,
  - (iii) a third zone capable of retarding the rate of migration of the sample and the non-immobilised molecule, and
  - (iv) a fourth zone comprising in at least a part of the fourth zone the same type of analyte as the one to be assayed or an analogue thereof in an immobilised state, which is capable of specifically binding to the non-immobilised molecule, and
  - (v) a calibration zone comprising an immobilised binding agent having an affinity for the labelled non-immobilised molecule capable of specifically binding to the analyte to be assayed.

wherein the amount of the analyte present in the sample is calculated from a signal obtained in the fourth zone and a signal obtained in the calibration zone.

2. (Currently Amended) [A] The device according to claim 1 wherein the first zone and the second zone are overlapping.
3. (Currently Amended) [A] The device according to any of the preceding claims wherein the second zone and the third zone are overlapping.

4. (Currently Amended) [A] The device according to claim 1 wherein the third zone and the fourth zone are overlapping.

5. (Currently Amended) [A] The device according to claim 1 wherein the device comprises a fifth zone into which a sample not detained during migration may be adsorbed.

6. (Currently Amended) [A] The device according to claim 5 wherein the fourth zone and the fifth zone are overlapping.

7. (Currently Amended) [A] The device according to claim 1 wherein the second zone comprises a non-immobilised second molecule capable of binding to a compound different from the analyte to be assayed and incapable of specifically binding to the analyte to be assayed, said second molecule provided with a detectable label.

8. (Currently Amended) [A] The device according to claim 7 wherein the fourth zone includes, in at least a part of the fourth zone in an immobilised state a compound different from the analyte to be assayed and capable of binding the non-immobilised second molecule, said compound incapable of binding specifically to the non-immobilised molecule capable of specifically binding to the analyte.

9. (Currently Amended) [A] The device according claim 1 wherein the non-immobilised molecule in the second zone is selected from the group consisting of antibodies, receptors and a combination thereof.

10. (Currently Amended) [A] The device according to claim 5 wherein the first zone, the second zone, the third zone, the fourth zone and the fifth zone is comprised of a porous material, selected from the group consisting of a nitrocellulose membrane, cellulose, a polymer, glass fibre, woven fibres, non-woven fibres and a chromatographic gel membrane, providing that each of the first zone, the second zone, the third zone, the

APPLN. NO.: 10/659,367

ATTORNEY DOCKET: 60589-000014

RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

fourth zone or the fifth zone is comprised of the same or different porous material as at least one of the remaining zones.

11. (Currently Amended) [A] The device according to claim 10 wherein the average pore size of the porous material is in the range of 10-10,000 nm.

12. (Currently Amended) [A] The device according to claim 10 wherein the capacity of the porous material to bind proteins is in the range of 1-400  $\mu\text{g}/\text{cm}^2$ .

13. (Currently Amended) [A] The device according to claim 10 wherein the capillary flow-rate of the porous material is in the range of 50-250 sec/4 cm.

14. (Currently Amended) [A] The device according claim 1 wherein the analyte or analogue is immobilised to the fourth zone through a spacer molecule.

15. (Currently Amended) [A] The device according to claim 14 wherein the spacer molecule includes a peptide, a polypeptide or a protein.

16. (Currently Amended) [A] The device according to claim 14 wherein the spacer molecule is bovine serum albumin.

17. (Currently Amended) [A] The device according to claim 15 wherein the spacer molecule and the analyte or analogue being immobilised to the fourth zone are coupled using carboxymethyloxime (CMO) and/or hemosuccinyl iodohistamine (HMS).

18. (Currently Amended) [A] The device according to claim 1 wherein the capability of retarding the sample and the non-immobilised molecule of the third zone is provided by changing the length of the porous material used in the third zone, changing the porosity of the porous material, adding at least one substance or a combination thereof.

APPL. NO.: 10/659,367

ATTORNEY DOCKET: 60589-000014

RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

19. (Currently Amended) [A] The device according to claim 18 wherein the sample and the non-immobilised molecule is retarded by changing the length of the third zone relative to the length of the first, second and fourth zones.

20. (Currently Amended) [A] The device according to claim 1 wherein the third zone comprises 1-99% of the device ~~porous material used in the first zone, second zone, third zone and fourth zone.~~

21. (Cancelled)

22. (Currently Amended) [A] The device according to claim ~~[[21]]~~ 1 wherein the calibration zone is located downstream from the fourth zone and upstream from the fifth zone.

23. (Currently Amended) [A] The device according to claim 20 wherein the calibration zone has immobilised thereon polyclonal or monoclonal antisera specific for the labeled non-immobilised molecule capable of binding the analyte to be assayed.

24. (Currently Amended) [A] The device according to claim 5 wherein at least one of the first zone, the second zone, the third zone, the fourth zone or the fifth zone includes at least one ancillary compound capable of improving the flow of the liquid sample.

25. (Currently Amended) [A] The device according to claim 24 wherein the at least one ancillary compound is a liquid.

26. (Currently Amended) [A] The device according to claim 24 wherein the at least one ancillary compound decreases non specific binding of the analyte and non specific binding of the non-immobilised molecule.

APPLN. NO.: 10/659,367

ATTORNEY DOCKET: 60589-000014

RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

27. (Currently Amended) [A] The device according to claim 24 wherein the at least one ancillary compound provides a substantially consistent and quantitative release of the non-immobilised molecule.

28. (Currently Amended) [A] The device according to claim 24 wherein the at least one ancillary compound provides low affinity for protein binding.

29. (Currently Amended) [A] The device according to claim 24 wherein the at least one ancillary compound provides low retention of triglyceride rich samples.

30. (Currently Amended) [A] The device according to claim 24 wherein the at least one ancillary compound decreases the viscosity of the sample.

31. (Currently Amended) [A] The device according to claim 24 wherein the at least one ancillary compound comprises chemical constituents including water, surfactant, salt, acid, base, metals, sugar, proteins or lipid.

32. (Currently Amended) [A] The device according to claim 1 wherein the device comprises a solid support.

33. (Currently Amended) [A] The device according to claim 1 wherein said device is provided in the form of a dry stick.

34. (Withdrawn) An appliance carrying a multiplicity of the devices according to any of claims 1, 5, 21 or 24.

35. (Withdrawn) An appliance according to claim 34 wherein an automatic, a semi-automatic or a continuous system is provided.

36. (Withdrawn) An appliance according to claim 34 wherein the appliance is a strip.

APPLN. NO.: 10/659,367

ATTORNEY DOCKET: 60589-000014

RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

37. (Withdrawn) A method for assaying an analyte in a sample, said method comprising:

- (i) applying the sample suspected of containing an analyte to a first zone,
- (ii) permitting the sample to migrate through a second zone comprising a non-immobilised molecule capable of specifically binding to the analyte, said non-immobilised molecule including a detectable label,
- (iii) permitting the sample to migrate through a third zone capable of retarding the rate of migration of the sample and the non-immobilised molecule, and
- (iv) permitting the sample to migrate through a fourth zone comprising in at least a part of the fourth zone the same type of analyte as the one to be assayed or an analogue thereof, in an immobilised state, which is capable of specifically binding to the non-immobilised molecule.

38. (Withdrawn) A method according to claim 37 wherein at least one ancillary compound capable of improving the flow of the sample is added.

39. (Withdrawn) A method according to claim 37 wherein at least one ancillary compound is incorporated into at least one of the first zone, the second zone, the third zone or the fourth zone.

40. (Withdrawn) A method according to claim 38 wherein the ancillary compound decreases non-specific binding of the analyte and non-specific binding of the non-immobilised molecule.

41. (Withdrawn) A method according to claim 38 wherein the ancillary compound provides a substantially consistent and quantitative release of the non-immobilised specific binding molecule.

42. (Withdrawn) A method according to claim 38 wherein the ancillary compound provides low affinity for protein binding.

APPLN. NO.: 10/859,367  
ATTORNEY DOCKET: 60589-000014  
RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

43. (Withdrawn) A method according to claim 38 wherein the ancillary compound provides low retention of triglyceride rich samples.
44. (Withdrawn) A method according to claim 38 wherein the ancillary compound decreases the viscosity of the sample.
45. (Withdrawn) A method according to claim 38 wherein the ancillary compound comprises chemical constituents selected from the group consisting of water, surfactant, salt, acid, base, metals, sugar, proteins and lipid.
46. (Withdrawn) A method according to claim 37 wherein the analyte to be assayed is a steroid selected from the group consisting of a progestagen, an estrogen and an androgen.
47. (Withdrawn) A method according to claim 46 wherein the progestagen to be assayed is progesterone.
48. (Withdrawn) A method according to claim 47 wherein the sample to be assayed comprises 0-50 ng/ml of progesterone.
49. (Withdrawn) A method according to claim 37 wherein the non-immobilised molecule is selected from the group consisting of antibodies and receptors.
50. (Withdrawn) A method according to claim 49 wherein the antibodies are monoclonal antibodies.
51. (Withdrawn) A method according to claim 69 wherein the first zone, the second zone, the third zone, the fourth zone and the fifth zone is comprised of a porous material, selected from the group consisting of a nitrocellulose membrane, cellulose, a polymer, glass fibre, woven fibres, non-woven fibres and a chromatographic gel

APPLN. NO.: 10/659,367

ATTORNEY DOCKET: 60589-000014

RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

membrane, providing that each of the first zone, the second zone, the second zone, the third zone, the fourth zone, or the fifth zone is comprised of the same or different porous material as at least one of the remaining zones.

52. (Withdrawn) A method according to claim 51 wherein the average pore size of the porous material is in the range of 10-10,000 nm.

53. (Withdrawn) A method according to claim 51 wherein the capacity of the porous material to bind proteins is in the range of 1-400  $\mu\text{g}/\text{cm}^2$ .

54. (Withdrawn) A method according to claim 51 wherein the capillary flow-rate of the porous material is in the range of 50-250 sec/4 cm.

55. (Withdrawn) A method according to claim 37 wherein the detectable label is selected from the group consisting of dyes, enzymes, fluorescent compounds, chemiluminescent compounds, radioactive labels and metals.

56. (Withdrawn) A method according to claim 55 wherein the detectable label is selected from the group consisting of gold, silver, carbon, fluorescent latex beads and dyed latex beads.

57. (Withdrawn) A method according to claim 37 wherein the assay time is less than 15 min.

58. (Withdrawn) A method according to claim 37 wherein the sample to be assayed is a mammalian physiological fluid.

59. (Withdrawn) A method according to claim 58, wherein the mammalian physiological fluid to be assayed is selected from the group consisting of milk samples, urinary samples, blood samples and saliva samples.



APPLN. NO.: 10/659,367

ATTORNEY DOCKET: 60589-000014

RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

60. (Withdrawn) A method according to claim 58 wherein the mammal is a cow or a human.

61. (Withdrawn) A method according to claim 37, utilizing a device according to claim 1 or an appliance according to claim 34.

62. (Withdrawn) A method according to claim 37 wherein the capability of retarding the sample and the non-immobilised molecule of the third zone is provided by changing the length of the porous material used in the third zone, changing the porosity of the porous material, adding at least one substance or a combination thereof.

63. (Withdrawn) A method according to claim 62 wherein the sample and the non-immobilised molecule is retarded by changing the length of the third zone relative to the length of the first, second and fourth zones.

64. (Withdrawn) A method according to claim 37 wherein the third zone comprises 1-99% of the porous material used in the first zone, the second zone, the third zone and the fourth zone.

65. (Withdrawn) A method according to claim 37 wherein the method further comprises passing the sample to a calibration zone.

66. (Withdrawn) A method according to claim 69 wherein the method further comprises passing the sample to a calibration zone located downstream from the fourth zone and upstream from the fifth zone.

67. (Withdrawn) A method according to claim 65 wherein the calibration zone has immobilised thereon polyclonal or monoclonal antisera specific for the labeled non-immobilised molecule capable of binding the analyte to be assayed.

APPLN. NO.: 10/659,367

ATTORNEY DOCKET: 60589-000014

RESPONSE AND AMENDMENT DATED: SEPTEMBER 11, 2006

68. (Currently Amended) [A] The device according to claim 10 wherein the polymer is nylon, polyvinylidene fluoride or latex.

69. (Withdrawn) A method according to claim 37 further comprising passing a sample not detained during migration to a fifth zone wherein the sample is absorbed.

70. (Withdrawn) A method according to claim 51 wherein the polymer is nylon, polyvinylidene fluoride or latex.